EXHIBIT 8

ELSEVIER

Biomaterials 23 (2002) 3487–3493

Biomaterials

www.elsevier.com/locate/biomaterials

PVDF as a new polymer for the construction of surgical meshes ☆

U. Klinge[a,*], B. Klosterhalfen[b], A.P. Öttinger[c], K. Junge[a], V. Schumpelick[a]

[a] *Department of Surgery, Technical University of Aachen, Pauwelsstraße 30, 52057 Aachen, Germany*
[b] *Institute for Pathology of the Rheinish Westfalian Technical University Aachen, Germany and Interdisciplinary Center for Clinical Research IZKF "BIOMAT", Germany*
[c] *Joint Insitute for Surgical research Aachen, Moscow, Russia*

Received 1 August 2001; accepted 14 February 2002

Abstract

Abdominal hernia repair is the most frequently performed operation in surgery. Mostly due to lowered recurrence rates mesh repairs in hernia surgery have become an integral component despite increasing mesh-related complications. Current available mesh prosthesis are made of polypropylene (PP), polyethylene-terephtalat or polytetrafluorethylene, though all of them reveal some disadvantages. The introduction of new materials seems to be advisable. Caused by supposed advantageous textile properties and tissue response two mesh modifications made of polyvinylidene fluoride (PVDF) for abdominal hernia repair were developed. In the present study the PVDF meshes were compared to a common heavy weight PP-mesh (Prolene®) in regard to functional consequences and morphological tissue response. After implantation in rats as inlay for 3, 14, 21, 42 and 90 days abdominal wall mobility was recorded by three-dimensional photogrammetry. Tensile strength of the suture zone and the mesh itself were determined. Explanted tissue samples have been investigated for their histological reaction in regard to the inflammatory infiltrate, vascularisation, connective and fat tissue ingrowth. Number of granulocytes, macrophages, fibroblasts, lymphocytes and foreign giant body cells have been evaluated to reflect quality of tissue response. The cellular response was grasped by measurement of DNA strand breaks and apoptosis (TUNEL), proliferation (Ki67) and cell stress (HSP70). Analyzing the results confirmed that construction of hernia meshes made of PVDF could be an advantageous alternative to the commonly used materials due to an improved biostability, lowered bending stiffness and a minimum tissue response.

Keywords: PVDF; Polymer; Surgical mesh; Hernia

1. Introduction

Actually, mesh repair of abdominal wall hernias has become an integral component of surgery, in several countries even representing the standard procedure. Meanwhile, about 1 million meshes are implanted world-wide per year. The extended implantation of alloplastic material in the flexible frame of muscles and fascial tissue is known to cause specific mesh-related complications like restriction of the abdominal wall mobility, complaints [1,2], induction of intra-abdominal adhesions with erosion of adjacent organs or consecutive fistula formation [3,4] to the bladder [5–7], bowel [8–11], vessels [12] and ductus deferens [13]. Next to an unavoidable inflammatory foreign body reaction (FBR) the prosthesis usually is embedded into a fibrous scar plate, which is responsible for a considerable shrinkage of the mesh area of about 40% [14,15] due to the physiological process of wound contraction. In particular, the enormous surface area of the implant affects the biocompatibility of the mesh material and represents a major factor for an appropriate incorporation into the host tissues.

Apart from the requirements on strength and elasticity it is the specific tissue response to the mesh-fibres that determines the suitability of the polymer. Current mesh prosthesis are made of polypropylene (PP), polyethylene-terephtalat (PET) or polytetrafluorethylene (PTFE), though all of them reveal some disadvantages. PET is supposed to be degraded after some years, which has been proven at vascular grafts made of PET [16–19]. Accordingly, first recurrences through disintegrated PET-meshes have been reported

☆ This study is performed in adherence to the *NIH* guidelines for the use of experimental animals and to the guidelines of the "*Deutsche Tierschutzgesetz*".

*Corresponding author. Tel.: +49-241-80-8089349; fax: +49-241-8888-415.

E-mail address: uklinge@post.klinikum.rwth-aachen.de (U. Klinge).

0142-9612/02/$ - see front matter
PII: S0142-9612(02)00070-4

[20]. PTFE is usually manufactured in the form of foils, thus lacking a sufficient integration into the surrounding tissue and, furthermore, is suspected to loose its stability as well [20–22]. PP is favoured for most of the mesh constructions. PP is still regarded as being stable for the long run, but it shows a comparatively high bending stiffness if used for mesh construction, resulting in rather stiff mesh prosthesis. However, as the main disadvantage the induction of a more accentuated inflammatory FBR [23] has to be regarded. As a result, all present polymers are not ideal to realize optimal mesh implants, either they do not allow appropriate textile constructions or they do not show a sufficient histological biocompatibility.

The polyvinylidene fluoride (PVDF) is a polymer with improved textile and biological properties [24]. In comparison to PET the PVDF is more resistant to hydrolysis and degradation. Furthermore, ageing does not increase the stiffness, evidently seen in PP. Although it has been introduced in vascular surgery since years, it has never been used for the construction of surgical meshes till now. For its suggested advantages we thus tested two mesh modifications made of PVDF fibres for their functional and histological characteristics in a standardized rat model.

2. Materials and methods

We investigated two mesh modifications out of PVDF monofilaments with different weights (PVDF-A and PVDF-B) in comparison to a common PP-mesh (Prolene®), that has been examined in the same model (published by Klosterhalfen et al., 1998 [25]).

Both PVDF (Ethicon, Norderstedt, Germany) meshes were reduced in weight in comparison to the PP-mesh, despite the higher specific weight of PVDF with 1.8 g/cm^3 compared to 0.9 g/cm^3 for PP. The PVDF-A modification with the lowest weight was made of PVDF fibres of 6/0 size, whereas the PVDF-B mesh with its slightly increased weight is made of even thinner fibres (7/0). As a consequence, the filament surface rose from 119 mm^2/cm^2 mesh for the PVDF-A modification to 236 mm^2/cm^2 for the PVDF-B, respectively (Table 1, Fig. 1). The commonly used heavy weight PP-mesh (Prolene®, Ethicon, Norderstedt, Germany) served as standard for its functional and histological reactions, being typical for heavy-weight PP-meshes.

2.1. Surgical procedure

In totally 125 male Wistar rats (250–300 g) the three meshes were implanted for 3, 14, 21, 42 and 90 days. Anesthesia was achieved with an intraperitoneal application of a mixture of Rompun® 2% (0.4 mg/100 g body weight) and ketamine-hydrochloride 10% (10 mg/100 g body weight). After midline incision, a full-thickness defect was performed resecting the rectus muscles with peritoneum (except skin) en bloc, an area of 2 cm horizontally to 3 cm vertically 1.5 cm distal of the xiphoid. Each mesh (2 × 3 cm^2) was fixed as real abdominal wall replacement continuously in inlay position with 5/0 Prolene®. Skin closure with 3/0 silk continuous sutures. No antibiotic treatment was given before or during the experiments.

3D-stereography: After sacrificing the animals each time the bending stiffness of the abdominal wall was determined using a video-graphic method based on three-dimensional stereography. The method was recently described in detail [26].

Tensiometry: After determination of the abdominal wall mobility the tensile strength was measured using a tensiometer. Measurements were made on 2 cm wide strips obtained after excision of the whole mesh with the

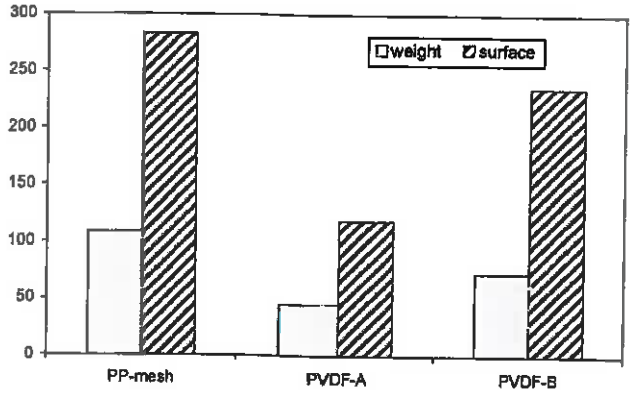

Fig. 1. Textile weight (g/m^2) and surface (mm^2/cm^2 mesh). The surface was calculated on the basis of the weight and the filament size.

Table 1
Textile characteristics

	PP-mesh	PVDF-A	PVDF-B
Polymer	PP	PVDF	PVDF
Filament size (g/1000 m)	21	10	7
Weight (g/m^2)	109	45	73
Surface (mm^2)	283	119	236
Length (cm/cm^2)	53	45	109

Table 2
Tensile strength (N/cm) of the mesh area after explantation, horizontal direction

Days after implantation	PP-mesh	PVDF-A	PVDF-B
3	89	21	18
14	84	37	25
21	86	34	23
42	—	31	20
90	80	33	31

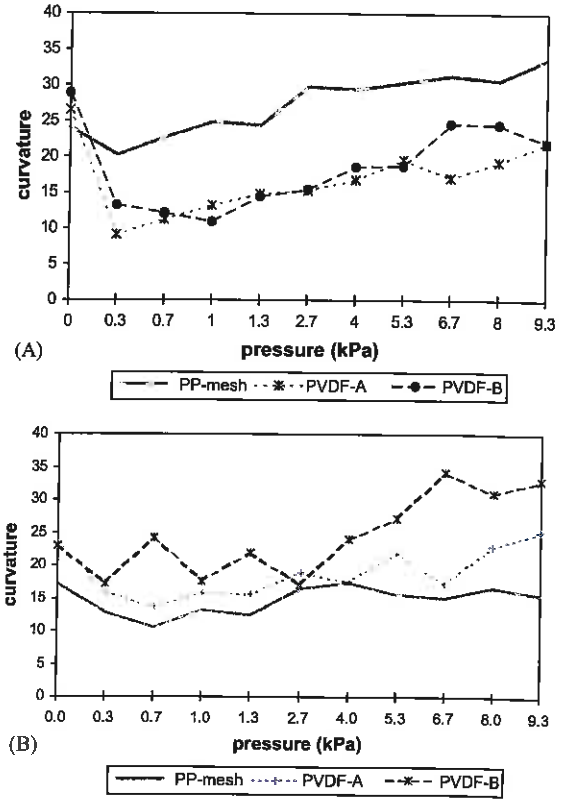

Fig. 2. Abdominal wall curvature 14 days (A) and 90 days (B) after mesh implantation in rats.

surrounding abdominal wall. The tests were carried out directly after explantation of each mesh. The tensile strength of the suture zone as well as that of the mesh alone were determined (velocity of stretching 1 cm/min).

2.2. Morphological study

Specimens were studied by light (LM)- and transmission electron microscopy (TEM). For LM tissue specimens were fixed in 10% formaldehyde, embedded in paraffin, and sections were stained with haematoxylin and eosin (H&E), as well as periodic-acid Schiff (PAS) plus diastase and elastica van Gieson (EvG). The morphometric evaluation consisted of a quantitative cell analysis of the inflammatory reaction and the soft-tissue reaction. It was performed both in the centre and the suture zone of the mesh. Cells in 10 fields of 5 HE slides at a grid of 10 points (140 ×, area 0.1 mm^2) and within the interface of 0–300 μm (area 636 μm^2) on the TEM were counted. Parameters measured were the inflammatory infiltrate (partial volume (PV) %), connective tissue (PV%), fat cells (PV%), vessels (PV%), macrophages (%), granulocytes (%), foreign body giant cells (%), lymphocytes (%) and fibroblasts (%).

Immunohistochemical investigations were performed on the material embedded in paraffin using the avidin-biotin-complex method and diaminobenzidine as a

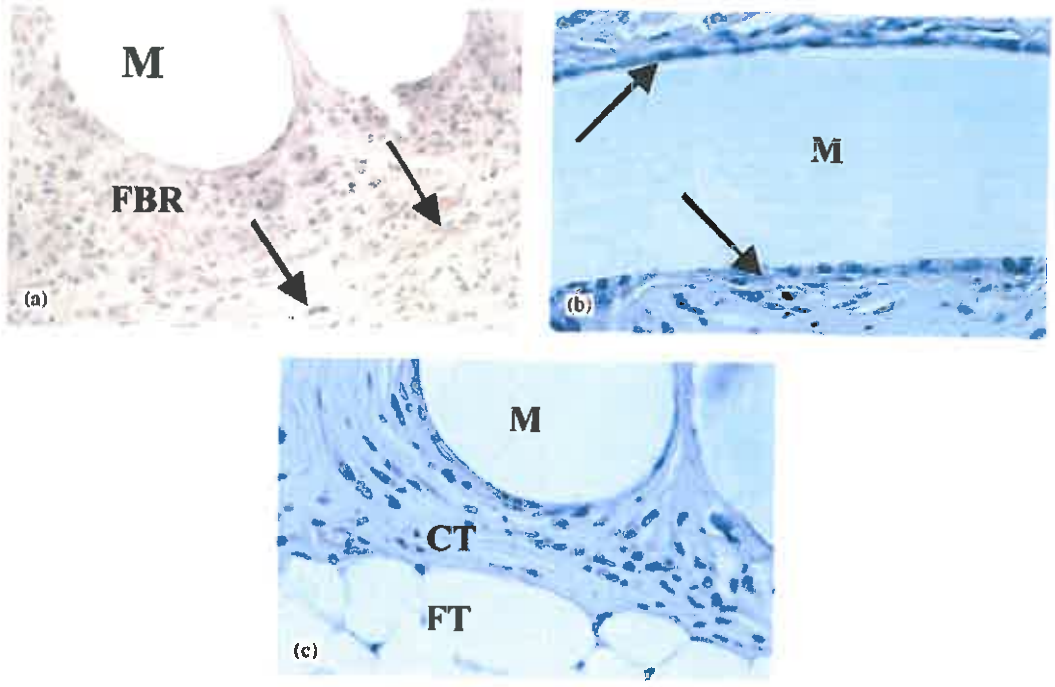

Fig. 3. (a): PP-mesh (HE, 250 ×, M = mesh); typical chronic FBR with numerous fibroblasts (arrows), 21 days after implantation (b): PVDF-A mesh (HE, 630 ×, M = mesh); 90 days after implantation with an almost monolayer of macrophages (arrows) at the interface; (c) PVDF-B mesh (HE, 630 ×, M = mesh) with a discrete FBR, minimal fibrosis (CT) and fat tissue (FT).

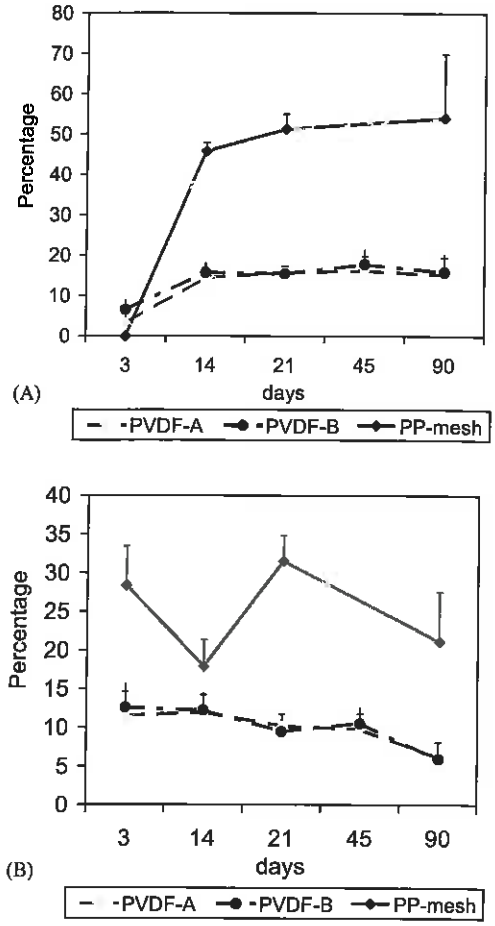

Fig. 4. Partial volume of inflammatory cells (A) and connective tissue (B) after mesh implantation in rats.

chromogen. Antibodies used in this study included polyclonal rabbit anti-heat shock protein 70 A500 (1:200, DAKO, Hamburg, Germany) and monoclonal HSP70/HSC70 SPA-820 for the cell stress response (1:200, BIOMOL, Hamburg, Germany), monoclonal mouse Ki67 (MIB5) for the cell proliferation rate (1:10, DIANOVA, Hamburg, Germany), as well as TUNEL for apoptosis and DNA-strand breaks (APOPTAG, Oncor, Hamburg, Germany).

2.3. Statistics

Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS®)—software. All functional and morphological results were analyzed for statistical significance using a corrected analysis of variance [least-significant-differences (LSD)-test] according to Bonferroni), followed by independent *t*-tests in case of significant differences; *p*-values <0.05 were considered to be significant. Analysis of categorical data were done by Chi-Square test.

3. Results

The clinical appearance of the animals was uneventfully, except for two deaths in the PP-group, one within the PVDF-A mesh group and four in the PVDF-B group that always occurred without any evident connection to the operation. Local signs of inflammation were seen in five animals with a PP-mesh, eight with a PVDF-A mesh and three with a PVDF-B mesh, respectively.

3.1. Functional properties

The tensile strength of the anchorage zone revealed no considerable differences between the three mesh variants. The tensile strength of the incorporated mesh remained constant over the whole time course and mirrors the distinct textile strength (Table 2).

The functional analysis of the abdominal wall curvature revealed a significant decrease for both PVDF meshes after 14 days of implantation. In contrast, at day 90 the curvature in both PVDF-groups, in particular PVDF-B, exceeded those of the PP-group (Fig. 2).

3.2. Tissue response

In contrast to the tissue response to heavyweight PP-meshes with their initial accumulation of monocytes, its comparatively thick granulomas and intensive fibrosis (Fig. 3a) throughout the whole pores, the inflammatory and fibrous tissue reaction to the PVDF modifications was significantly decreased. The frequently seen small free spaces around the polymer reflected the loose contact between fibres and surrounding cells. A remarkable number of fibroblasts did not appear before 21 days after implantation, forming a thin connective tissue capsule around the polymer fibres (Fig. 3b). Although with its comparatively increased amount of material, the tissue reaction to the PVDF-B mesh was similar to that of the PVDF-A modification. Both mesh-modifications induced only rudimentary signs of inflammatory reaction. The fibrosis was limited to a small area around the fibres with pores filled with fat tissue (Fig. 3c).

The morphometric analysis of the partial volume of inflammatory cells revealed similarly a significant reduction for both PVDF meshes (Fig. 4A) with a gradual decrease to the end of the observation period. This inflammatory reaction corresponded to the extent of fibrosis (Fig. 4B) that, in contrast, always remained constant from day 14 to day 90. Whereas numerous granulocytes were seen at the interface of the PP-mesh with a decline over time, this cell type was only rarely seen within both PVDF mesh groups (Fig. 5A). For these materials the number of macrophages showed a peak after 45 days followed by a slight decrease to day 90. In contrast, the PP-mesh always indicated an

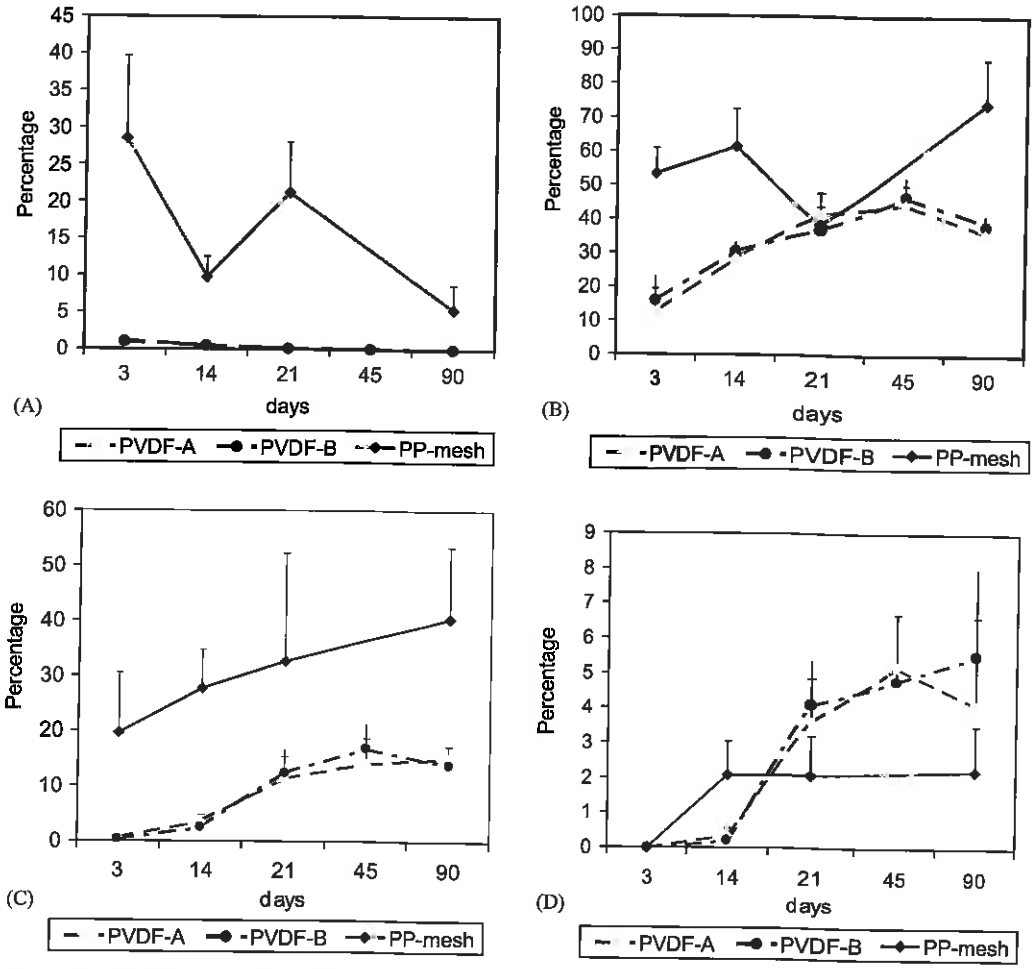

Fig. 5. Number of granulocytes (A), macrophages (B), fibroblasts (C) and foreign body giant cells (D) at the interface after mesh implantation in rats.

increased number of macrophages compared to both PVDF mesh-modifications and a continuous rise over the whole time course (Fig. 5B). This distinct accumulation of macrophages was closely mirrored by the number of fibroblasts (Fig. 5C), whereas the course of foreign body giant cells runs inversely (Fig. 5D).

The cellular response showed a constantly decreased level of TUNEL positive cells for both PVDF meshes, indicating a lowered rate of apoptosis and DNA strand brakes (Fig. 6A), whereas Ki 67 positive cells were increased within the first weeks but reduced after 6 and 12 weeks (Fig. 6B), almost reaching a physiological level. In contrast, the expression of heat shock protein HSP 70 as an indicator for cellular stress always showed significant higher values for the PVDF mesh groups. After 14 days levels constantly rose until day 90 (Fig. 6C).

Though considerable differences of weight both PVDF-modifications showed quite similar reactions in regard to their functional, histological and cellular properties.

4. Discussion

In hernia surgery the primary task of biomaterials is the durable and adequate reinforcement of the abdominal wall. This request demands the absence of any polymer degradation as well as a textile construction that prevents any functional and histological impairment. Because of the known disadvantages of PET, PP and PTFE, the introduction of new polymers seems to be advisable.

Recent experiences of the cadiovascular surgeons with a PVDF suture (PVDF, Teflene®) underlined the excellent textile properties in vitro [27]. Textile analysis measuring the tensile strength, the elongation, the surface roughness, the bending stiff and tissue drag revealed equivalent characteristics as found for common PP-sutures. In animal experiments for up to 2 years, there was visual evidence of surface stress cracking for PP but not for PVDF [28]. Even after 9 years of implantation the PVDF preserved its mechanical stability with 92.5% of its original strength [29]. In

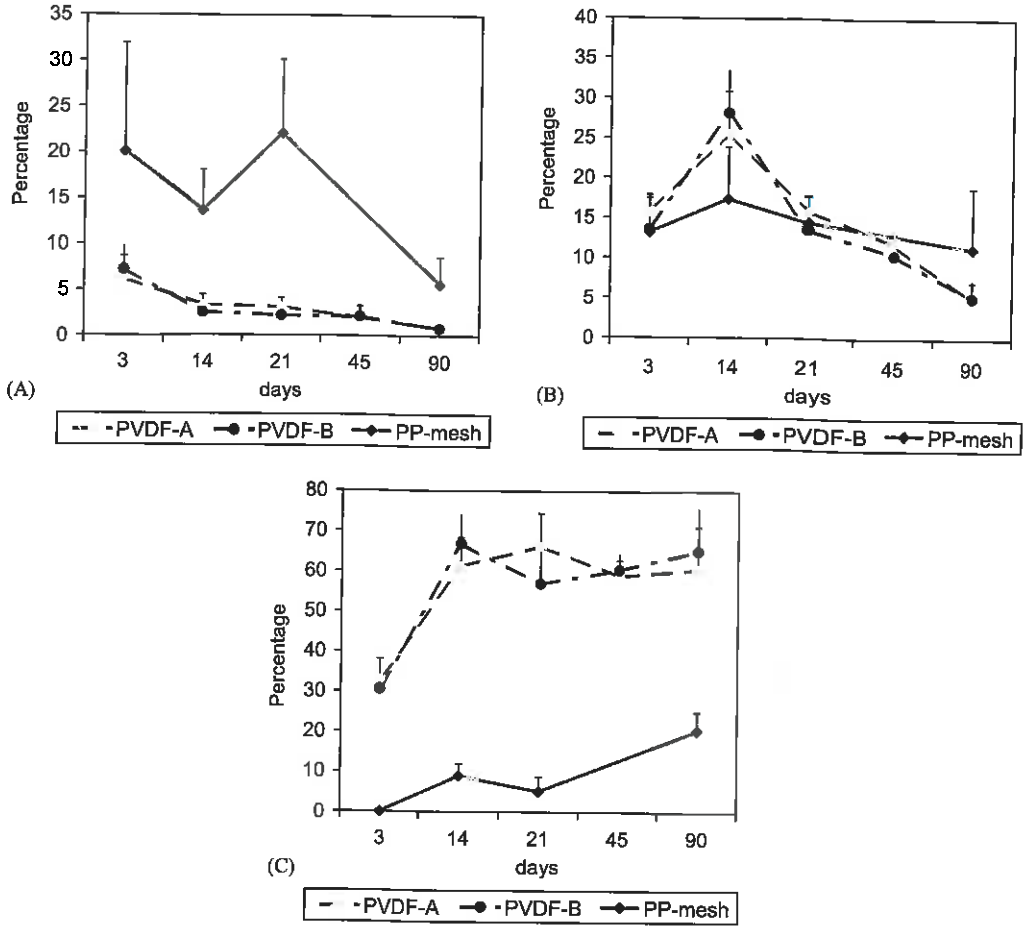

Fig. 6. Number of TUNEL positive (A), Ki 67 positive (B) and HSP 70 expressing cells (C) at the interface after mesh implantation in rats.

contrast, the PP-sutures lost about 46.6% of their stability, due to an accumulation of oxidation byproducts and water molecules near the surface. The histological examination of sutures made of PVDF after incorporation for 6 months in dogs showed only minimal cellular response without excessive fibrous tissue reaction [24,30].

The present study confirms for the first time that a mesh construction based on PVDF-monofilaments is feasible. Although the textile and filament properties can still be improved, for example, by enlarging the pore size or using more flexible fibres, the present modifications already showed a sufficient functional result, avoiding the increased abdominal wall stiffness seen 90 days after PP-mesh implantation. The reduction of the curvature with the PVDF materials after 14 days is suggested to be the consequence of the comparatively small pores, favouring a temporary decrease of flexibility.

However, in comparison with the PP the analysis of the tissue reaction to the PVDF clearly showed an improved biocompatibility that was slightly affected by the amount of material. Both PVDF-modifications induced significantly lower inflammation and fibrosis. The collagenous capsule was always limited to the perifilamentary region, not producing a scar plate, which embeds the entire mesh, which is typical for the PP-mesh. The moderate inflammatory activity of the FBR was characterized by a reduced accumulation of granulocytes, macrophages and fibroblasts with a predominance of foreign body giant cells. Giant cells represent the maturing of the granulomas indicating a primarily chronic course. The reduced cell activation is mirrored by the low rate of apoptosis and proliferation at the end of the observation period, almost reaching physiological levels [31]. However, both PVDF-meshes revealed significantly more HSP 70 expressing cells at the interface compared to PP. HSP 70 is known to be an indicator for cell stress, and furthermore, is supposed to be protective against cell damage. Accordingly, in 2000 Klosterhalfen et al. could demonstrate that the HSP 70 expression runs inversely to the inflammatory activity.

Altogether the histological analysis of the tissue reaction in rats confirmed the superior integration in comparison to a common PP-mesh. Whether this is mainly due to the reduced amount of material with a corresponding surface reduction or a specific result of

the polymer itself has to be investigated in further experimental studies. However, the present data clearly show that PVDF is a possible alternative to the established materials. In particular, the improved biostability, the lowered bending stiffness and the minimum tissue reaction at the interface favours the construction of hernia meshes to reduce mesh-related side effects.

Acknowledgements

This work was supported by BMBF Grant Project No. 01KS9503/9 for IZKF-BIOMAT, RWTH-Aachen, and ETHICON® in Norderstedt—Germany.

References

[1] McLanahan D, King LT, Weems C, Novotney M, Gibson K. Retrorectus prosthetic mesh repair of midline abdominal hernia. Am J Surg 1997;173(5):445–9.

[2] Vestweber K, Lepique F, Haaf F, Horatz M, Rink A. Results of recurrent abdominal wall hernia repair using polypropylene-Mesh. Zentralbl Chir 1997;122:885–8.

[3] Schneider R, Herrington Jr. JL, Granda AM. Marlex mesh in repair of a diaphragmatic defect later eroding into the distal esophagus and stomach. Am Surg 1979;45(5):337–9 [ISSN: 0003-1348].

[4] Fitzgerald PG, Walton JM. Intratracheal granuloma formation: a late complication of Marlex mesh splinting for tracheomalacia. J Pediatr Surg 1996;31(11):1568–9 [ISSN: 0022-3468].

[5] Houdelette P, Dumotier J, Berthod N, Peyrottes A. Urological surgical effect of the repair of inguinal hernia using bilateral subperitoneal prosthetic mesh. Ann Urol Paris 1991;25(3):138–41.

[6] Gray MR, Curtis JM, Elkington JS. Colovesical fistula after laparoscopic inguinal hernia repair. Br J Surg 1994;81(8):1213–4.

[7] Hume RH, Bour J. Mesh migration following laparoscopic inguinal hernia repair. J Laparoendosc Surg 1996;6(5):333–5.

[8] DeGuzman LJ, Nyhus LM, Yared G, Schlesinger PK. Colocutaneous fistula formation following polypropylene mesh placement for repair of a ventral hernia: diagnosis by colonoscopy. Endoscopy 1995;27(6):459–61.

[9] Kaufman Z, Engelberg M, Zager M. Fecal fistula: a late complication of Marlex mesh repair. Dis Colon Rectum 1981;24(7):543–4.

[10] Soler M, Verhaeghe P, Essomba A, Sevestre H, Stoppa R. Treatment of postoperative incisional hernias by a composite prosthesis (polyester-polyglactin 910). Clinical and experimental study. Ann Chir 1993;47(7):598–608.

[11] Miller K, Junger W. Ileocutaneous fistula formation following laparoscopic polypropylene mesh hernia repair. Surg Endosc 1997;11(7):772–3.

[12] Schumpelick V, Kingsnorth G. Incisional hernia of the abdominal wall, 1st ed. Berlin: Springer, 1999.

[13] Silich RC, McSherry CK. Spermatic granuloma. An uncommon complication of the tension-free hernia repair. Surg Endosc 1996;10(5):537–9.

[14] Amid P. Classification of biomaterials and their related complications in abdominal wall hernia surgery. Hernia 1997;1:5–8.

[15] Meddings RN, Carachi R, Gorham S, French DA. A new bioprosthesis in large abdominal wall defects. J Pediatr Surg 1993;28(5):660–3.

[16] Vinard E, Eloy R, Descotes J, et al. Stability of performances of vascular prostheses retrospective study of 22 cases of human implanted prostheses. J Biomed Mater Res 1988;22:633–48.

[17] Maarek J, Guidon R, Aubin M, Prud'homme R. Molecular weight characterization of virgin and explanted polyester arterial prosthesis. J Biomed Mater Res 1984;18:881–94.

[18] Berger K, Suavage L. Late fiber deterioration in Dacron arterial grafts. Ann Surg 1980;193(4):477–91.

[19] Riepe G, Loos J, Imig H, et al. Long-term in vivo alterations of polyester vascular grafts in humans. Eur J Vasc Endovasc Surg 1997;13(6):540–8.

[20] Schumpelick V, Wantz G. Inguinal hernia repair, 1st ed. Basel: Karger Verlag, 1995.

[21] Hauptmann S, Klosterhalfen B, Mittermayer C, Rühlmann K, Kaufmann R, Höcker H. Evidence for macrophage-mediated defluorization of a Teflon vascular graft. J Mater Sci: Mater Med 1996;7:345–8.

[22] Flessenkamper I, Muller KM. Biodegradation of a PTFE prosthesis. Zentralbl Chir 2001;126(2):151–5 [discussion 155–6].

[23] Klinge U, Klosterhalfen B, Conze J, et al. Modified mesh for hernia repair that is adapted to the physiology of the abdominal wall. Eur J Surg 1998;164(12):951–60.

[24] Urban E, King MW, Guidoin R, et al. Why make monofilament sutures out of polyvinylidene fluoride? ASAIO J 1994;40(2):145–56.

[25] Klosterhalfen B, Klinge U, Schumpelick V. Functional and morphological evaluation of different polypropylene-mesh modifications for abdominal wall repair. Biomaterials 1998;19:2235–46.

[26] Klinge U, Klosterhalfen B, Muller M, Anurov M, Ottinger A, Schumpelick V. Influence of polyglactin-coating on functional and morphological parameters of polypropylene-mesh modifications for abdominal wall repair. Biomaterials 1999;20(7):613–23.

[27] Hong T, King MW, Michielsen S, et al. Development of in vitro performance tests and evaluation of nonabsorbable monofilament sutures for cardiovascular surgery. ASAIO J 1998;44(6):776–85.

[28] Mary C, Marois Y, King M, et al. Comparison of the in-vivo behaviour of polyvinylidenfluoride and polypropylene sutures in vascular surgery. ASAIO J 1998;44:199–206.

[29] Laroche G, Marois Y, Schwarz E, et al. Polyvinylidene fluoride monofilament sutures: can they be used safely for long-term anastomoses in the thoracic aorta? Artif Organs 1995;19(11):1190–9.

[30] Laroche G, Marois Y, Guidoin R, et al. Polyvinylidene fluoride (PVDF) as a biomaterial: from polymeric raw material to monofilament vascular suture. J Biomed Mater Res 1995;29(12):1525–36.

[31] Akasaka Y, Ishikawa Y, Ono I, et al. Enhanced expression of caspase-3 in hypertrophic scars and keloid: induction of caspase-3 and apoptosis in keloid fibroblasts in vitro. Lab Invest 2000;80(3):345–57.